Desirable Characteristics of Forage Legumes for Improving Protein Utilization in Ruminants[1,2]

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ABSTRACT: Forages help meet the protein requirements of ruminants by providing degraded CP for microbial protein synthesis plus protein that escapes ruminal degradation. Evidence from numerous feeding studies with lactating dairy cows indicates that excessive ruminal protein degradation may be the most limiting nutritional factor in higher-quality temperate legume forages. Hence, there is interest in identifying factors that influence the rate and extent of ruminal degradation of forage proteins. Condensed tannins found in legumes are known to decrease protein degradation, either by altering the forage proteins or by inhibiting microbial proteases. Quadratic regressions of degradation rate and estimated protein escape on tannin concentration reached minimal rate (.048/h) and maximal escape (56%) at 27 g of tannic acid equivalents/kg of DM. Although most tannin-containing forages are not well-adapted to growing conditions in North America, biotechnology has been used to inject genes for tannins into adapted germplasm. The CP in red clover, which has no detectable tannins, was found to be less degradable than that in alfalfa, both in the silo and in the rumen. Small differences in protein degradability also were detected among alfalfa germplasm. Protein in alfalfa harvested as hay, rather than as silage, was used more efficiently for milk protein synthesis when fed to lactating cows; degraded CP from hay was captured more efficiently by ruminal microbes for protein synthesis in vitro. A ruminal escape of approximately 35% for total dietary CP is recommended by the NRC for lactating dairy cows fed mixed diets with 1.6 to 1.7 Mcal of NE_l/kg of DM. Ruminal degradation of CP from the forage portion of the diet can exceed 65% when forages are the major source of degradable protein. When ruminants obtain most or all of their nutrients from forage, the ruminal escape for forage protein should approximate 35%.

Key Words: Rumen Degraded Protein, Rumen Undegraded Protein, Microbial Proteins, Forage, Alfalfa, Maize Silage

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Introduction

Forage protein serves as a source of absorbed protein (**AP**) to the ruminant by providing both ruminal degraded protein (**RDP**) for microbial protein synthesis plus ruminal undegraded protein (**RUP**) that escapes microbial breakdown. Rapid and extensive ruminal degradation of proteins in legume and grass forages generally leads to decreased protein efficiency. Forage N is present as both protein and nonprotein N (**NPN**). High-quality silages often contain excessive amounts of NPN (Muck, 1987). Means (± SD) for alfalfa silage fed in 19 lactation trials were 54 (± 10)% NPN (total N basis) and 20.4 (± 1.4)% CP and 43 (± 5)% NDF (DM basis) for alfalfa harvested at an average 43 (± 9)% DM (G. A. Broderick, 1994, unpublished data). Forage NPN consists of oligopeptides, free amino acids, ammonium compounds, and other small molecules that rapidly contribute to the ruminal ammonia pool. The proportion of CP that is RDP and RUP in any forage depends on the properties of its protein. When protein degradation is very rapid, ruminal microbes cannot utilize all of the amino acids and ammonia released and more protein will be degraded than is synthesized. This net loss of protein and consequent loss of ammonia from the rumen is referred to as "ammonia overflow." Recent reviews on ruminal N metabolism have been published (Broderick et al., 1991; Wallace, 1994).

Increasingly, forages are being ensiled because of greater opportunity for mechanization and reduced labor requirements and increased speed of harvest and

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hence reduced chance of weather damage relative to harvesting hay. Dairy cows in early lactation have very high protein requirements; feeding dairy cows diets based on legume silages often necessitates supplementation with feeds high in RUP. The amount of microbial protein formed in the rumen is related to dietary DE rather than protein (Broderick and Merchen, 1992). However, there is a limit to the amount of DE that can be fed to dairy cows because of problems (e.g., low ruminal pH) that may result from high ration fermentability. There is a trend toward increased grazing of legume and grass pastures in the United States; N utilization by grazing ruminants is remarkably inefficient (Beever, 1982). Poor utilization of dietary N in ruminants, especially dairy cattle, has led to increased concern about the contribution of excreta to N pollution of the environment (Tamminga, 1992).

This review considers 1) theoretical calculations based on NRC (1989) tabular data suggesting why legume forage proteins are poorly utilized by lactating dairy cows, 2) recent evidence demonstrating low N utilization efficiencies for forages, 3) possible explanations for the inherently low N utilization efficiencies of forages, and 4) desirable characteristics for legume forages that have the potential to improve protein utilization in ruminants.

Theoretical Considerations

Some understanding of how altering the characteristics of forage legumes may be beneficial to protein utilization by lactating dairy cows may be derived from theoretical computations based on using NRC (1989) tables to formulate a diet with 28% NDF composed only of high-moisture corn plus alfalfa silage (Table 1). The NRC (1989) has tabulated the undegraded intake protein (**UIP**) content of alfalfa silage at 23% of total CP, a value that is 82% of the 28% UIP assigned to alfalfa hay. In vitro and in vivo findings suggest that the UIP of alfalfa silage may be nearer to 50% of that of alfalfa hay (Broderick et al., 1993b), or approximately 15% of total CP. Milk yields predicted from NRC (1989) computations with intakes of the diet in Table 1 ranging from 20 to 25 kg/d, assuming the UIP of alfalfa silage equaled 15, 23, or 30% of CP and milk yield was limited by supply of NE_l or AP, are shown in Figure 1. When alfalfa silage was assigned a UIP of 23% (NRC, 1989), milk yields predicted from NE_l and AP supply at DMI of 20 kg/d differed by 1.4 kg/d; at DMI of 25 kg/d, NE_l supply was sufficient for 5.2 kg/d more milk than AP supply. However, if the UIP of alfalfa silage were only 15%, then milk yield predicted from AP supply would range from 5.2 to 9.9 kg/d less than that predicted from NE_l supply at DMI ranging from 20 to 25 kg/d (Figure 1). If the UIP content of alfalfa silage could be doubled from the presumptive value of 15% of CP to 30% of CP, without altering NE_l content, then production predicted from AP supply on this diet would be improved substantially: Milk yield at a DMI of 20 kg/d predicted from AP supply actually would be 1.9 kg/d greater than that predicted from NE_l supply, with little difference between yield predicted from AP and NE_l supplies at 22.5 and 25 kg/d of DMI (Figure 1).

The NRC (1985) developed regressions expressing microbial protein yields as a function of NE intakes in sheep, beef cattle, and dairy cattle; slope of the regression used for dairy cattle (NRC, 1989) is 11.45 g of microbial CP/Mcal of NE_l consumed. It is known that microbial protein yields per unit energy fermented vary in vitro with pH (Strobel and Russell, 1986) and with supply of peptides (Argyle and Baldwin, 1989) and degraded protein (Stokes et al., 1991; Hristov and Broderick, 1994), and in vivo with ruminal dilution rate (Owens and Goetsch, 1986) and feeding frequency (Cecava et al., 1990). If microbial protein yields were increased by 10% (i.e., if slope of the regression equaled 12.6 g of microbial CP/Mcal of NE_l), then the milk production predicted from AP supply on the alfalfa silage (UIP assumed equal to 15%) plus high-moisture corn diet would be increased, relative to the microbial CP yield maintained at 100% of the NRC standard, by 3.7, 4.1, and 4.6 kg/d at DMI of 20.0, 22.5, and 25 kg/d, respectively.

Evidence of Poor Utilization of Protein in Legume Forages

In an experiment designed to study forage energy content, lactating cows were fed alfalfa silage, alfalfa hay, or two levels of corn silage as their sole forage (Broderick, 1985). Despite differences in NE_l content, feeding diets with approximately 60% of DM from alfalfa forages or corn silage resulted in similar yields

Table 1. Composition of a diet formulated to 28% NDF from alfalfa silage and high-moisture corn (NRC, 1989)

Item	Diet content
Component, % of DM	
Alfalfa silage (20% CP)	61.7
High-moisture corn (10% CP)	37.0
Mineral and vitamin supplements	1.3
Chemical composition	
CP, % of DM	16.0
NDF, % of DM	28.0
NE_l, Mcal/kg	1.66
UIP, % of CP[a]	26.9 (23% UIP)
	20.8 (15% UIP)

[a]Diet content of undegraded intake protein (UIP) computed assuming UIP content of high-moisture corn was 40% of CP and UIP content of alfalfa silage was either 23 or 15% of CP.

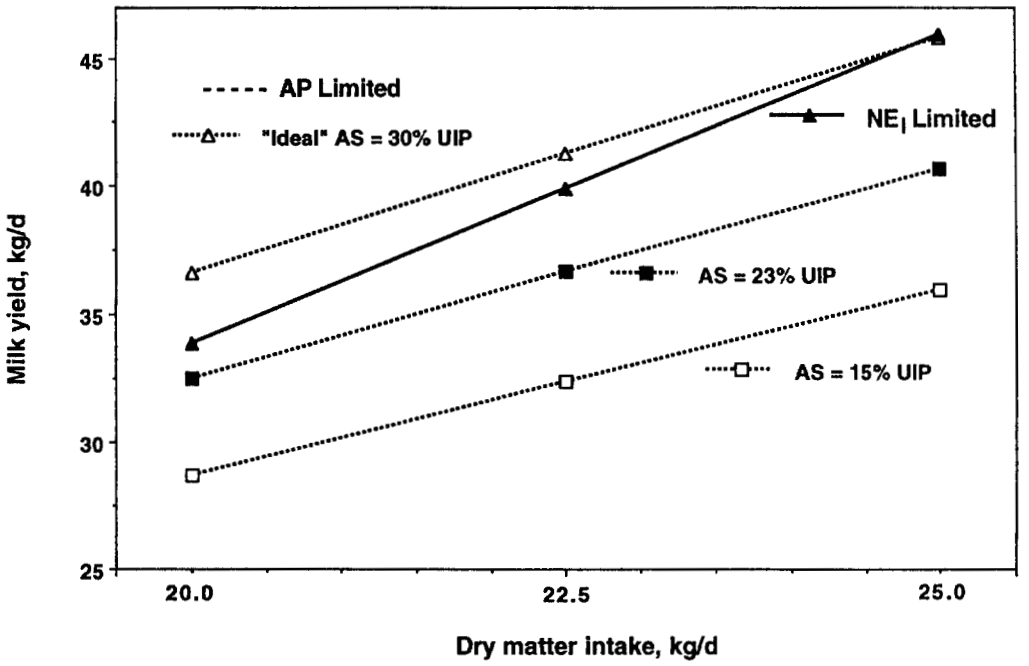

Figure 1. Estimated milk yields at DMI ranging from 20 to 25 kg/d on a diet formulated from alfalfa silage and high-moisture corn to 28% NDF and assuming production is limited by supply of NE_l or absorbable protein (AP). Limitation of milk yield by AP computed assuming that alfalfa silage undegraded intake protein (UIP) content was 15, 23, or 30% of CP.

of milk and fat; milk and fat yields were lower when corn silage was fed at 76% of DM in an attempt to provide NE_l equal to that on the alfalfa forages (Table 2). However, cows fed alfalfa silage or hay produced milk with lower protein content than cows fed corn silage-based diets that were supplemented with soybean meal (**SBM**) to equalize dietary CP. Milk protein yield was significantly greater for cows fed the diet with 60% corn silage; proportion of dietary CP secreted as milk protein also was highest and, by difference, N loss in excreta was lowest on this diet (Broderick, 1985). Part of this effect probably was mediated through greater ruminal fermentability of corn silage resulting in greater microbial protein formation. Also, the RUP content of alfalfa forages apparently was lower than that of SBM, which is extensively degraded in the rumen (NRC, 1989).

Several lactation studies were conducted to test the hypothesis that it is protein that first limits milk production when high-quality alfalfa silage is fed.

Table 2. Effect of feeding alfalfa silage (AS), alfalfa hay (AH), or corn silage (CS) as the sole dietary forage in lactating cows (Broderick, 1985)

Item	63%[a] AS	60% AH	60% CS	76% CS	SEM
Dietary CP, % of DM	17.7	16.5	16.5	16.7	—
Dietary NE_l, Mcal/kg of DM[b]	1.50	1.49	1.67	1.60	—
DMI, kg/d	24.1	24.0	23.1	23.9	.6
NE_l intake, Mcal/d	36.2	35.8	38.5	38.1	—
Milk, kg/d	29.8^c	29.4^{cd}	30.3^c	28.0^d	.6
Milk fat, %	3.68	3.70	3.86	3.84	.11
Milk fat, kg/d	1.09^{cd}	1.08^{cd}	1.14^c	1.05^d	.02
Milk protein, %	3.11^d	3.11^d	3.32^c	3.33^c	.04
Milk protein, kg/d	.92^d	.91^d	.99^c	.91^d	.02
Milk N/N intake	.211	.225	.254	.223	—

[a]Proportion of dietary DM coming from the respective forages.
[b]NE_l contents of diets recalculated using the equations of Mertens (1987) to compute NE_l from ADF in alfalfa forages and corn silage.
[c,d]Means in rows with different superscripts differ ($P < .05$).

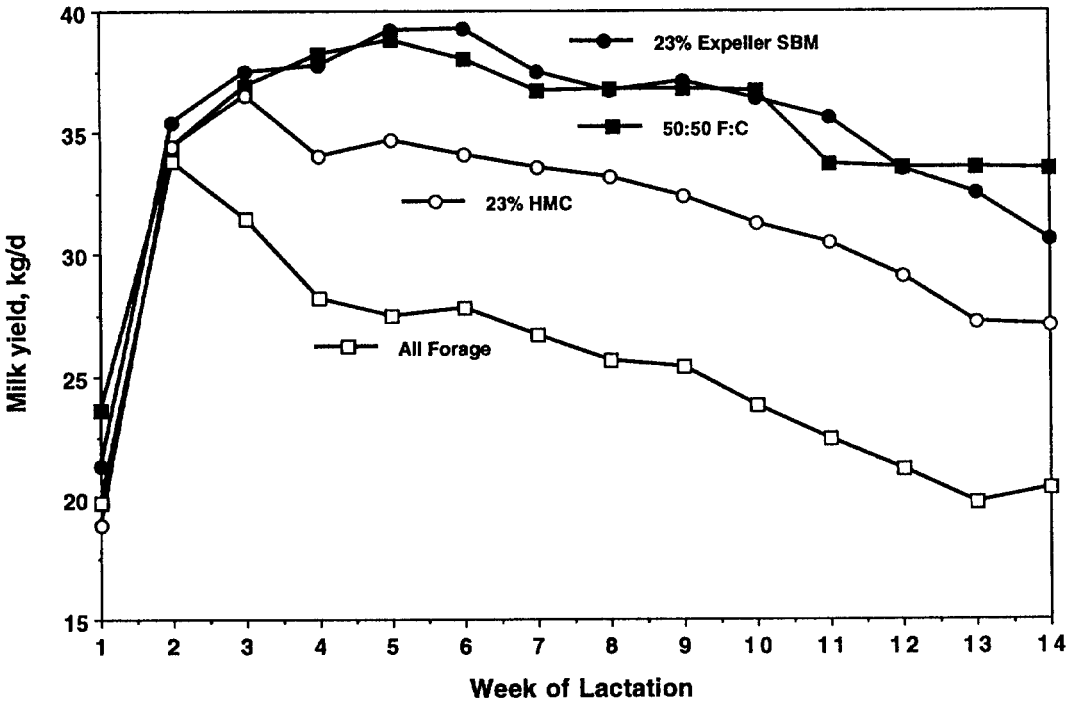

Figure 2. Milk yield from wk 1 to 14 of lactation of cows fed diets that were all alfalfa silage (All Forage), 23% high-moisture corn plus alfalfa silage (23% HMC), 23% expeller SBM plus alfalfa silage (23% Expeller SBM), or 50% alfalfa silage, 40% high-moisture corn plus 10% solvent SBM (50:50 F:C) (Cadorniga and Satter, 1993).

Energy also may be limiting on high-forage diets, but the purpose of the experiments was to determine whether an improved AP supply on alfalfa silage-based diets would increase milk production, even when dietary CP exceeded NRC recommendations. Beginning 14 d after calving, Dhiman et al. (1993) fed multiparous cows one of three diets: 1) 50% alfalfa silage, 40% high-moisture corn, and 10% solvent SBM (forage:concentrate ratio of 50:50); 2) 100% alfalfa silage supplemented with minerals and vitamins; 3) 100% alfalfa silage diet plus minerals and vitamins but supplemented with RUP by abomasal infusion of 1.0 kg of casein/d. As expected, production was greatest for cows fed diet 1. However, compared with diet 2, cows given the casein infusion yielded 6.9 kg/d more milk and .18 kg/d more protein. If energy were the first-limiting nutrient, then a large increase in milk yield with infusion of only 1.0 kg of casein/d would not have occurred.

A second trial (Cadorniga and Satter, 1993) quantified the milk yield response to supplementation of alfalfa silage-based diets with either energy (23% of DM as high-moisture corn) or RUP (23% of DM as expeller SBM with estimated UIP equal to 60% of CP). Positive and negative controls corresponding, respectively, to diets 1 and 2 of Dhiman et al. (1993) also were fed. Compared with the all-alfalfa-silage diet, milk yield was greater on all three supplemented diets (Figure 2). Yields of milk, fat, and protein were not different between cows fed the positive control and the 23% expeller SBM diet; yields of milk and milk components were lower on the diet with 23% high-moisture corn. Greater response to RUP than to energy supports the view that AP supply to the intestine is inadequate in dairy cows fed large amounts of high-protein legume silage. The observation that feeding protein resistant to ruminal degradation gave greater milk production than feeding an equal amount of high-moisture corn also suggests that diets containing large amounts of high-quality alfalfa forage are less limiting in energy than was previously thought.

A series of five Latin square feeding studies compared the milk protein response of multiparous dairy cows to RUP from either expeller SBM (Broderick et al., 1990) or fish meal (**FM**) protein (Broderick, 1992). Cows were fed diets containing 56 to 70% alfalfa silage (DM basis) and supplemented with high-moisture corn as the principal concentrate; solvent SBM served as the standard protein supplement. Response was computed as the increase in milk protein yield with supplemental protein, compared with the negative control (only alfalfa silage plus high-moisture corn). The response to solvent SBM was set equal to 1.0. Relative response to expeller SBM in the three trials was 1.48 (i.e., a mean increase in protein yield 48% greater than that with solvent SBM). The mean relative response to low solubles FM

in two trials was 2.07. Mean relative response in one trial to high solubles FM was 1.56, comparable to that with expeller SBM.

Faldet and Satter (1991) compared raw soybeans and roasted soybeans to solvent SBM in multiparous cows from d 15 to 120 of lactation. The roasted soybeans were heated to 146°C, then held at 120 to 125°C for 30 min before they were allowed to cool. Diets contained (DM basis) 50% alfalfa silage and 50% concentrate consisting of ground shelled corn and vitamin-mineral mix plus equal CP from one of the three supplemental proteins to provide 19% dietary CP. The roasted soybean diet, although containing the same amount of energy as that with raw soybeans, supported 4.7 kg/d greater milk yield. Milk yield was approximately equal on solvent SBM and raw soybeans, suggesting the benefit from roasted soybeans was due to RUP and not energy. Similar results were obtained by Voss et al. (1988) when solvent SBM was compared with roasted soybeans in a 42-d lactation study. It is important to note that the roasted soybeans fed in these trials were exposed to more extensive heat treatment than is often used for commercially roasted soybeans.

If heated appropriately, RUP content of forages can be increased in much the same way as protein concentrates. Forage DM content at time of ensiling has substantial influence on protein utilization via effect on extent of heating in the silo. Intestinal flow and digestion of nonammonia N (**NAN**) was measured in cows fed diets containing 35% of DM from concentrate and 65% from alfalfa conserved as silage at three different DM levels (Merchen and Satter, 1983). Feeding diets with alfalfa silage containing 29, 40, or 66% DM, and with daily N intake of 476, 542, or 501 g/d, resulted in NAN flow to the intestine of 326, 393, or 463 g/d, and NAN digestion in the small intestine equivalent to 43, 44, or 59% of the CP intake. Roffler and Satter (1985) compared alfalfa ensiled at 44% DM (high-moisture) or 64% DM (low-moisture) to dehydrated alfalfa by measuring their effect on plasma concentrations of branch-chain amino acids (**BCAA**) in yearling heifers. Plasma BCAA are an indicator of amino acid uptake from the intestine (Bergen, 1979). Animals were fed diets with (DM basis) 20% corn grain and 80% forage. As alfalfa was increased from 0 to 80% of dietary DM (by replacing corn silage), plasma BCAA increased 74, 227, and 298 nmol/mL with feeding of high-moisture silage, low-moisture silage, and dehydrated alfalfa, respectively. Greater elevation of BCAA is evidence that dehydrated alfalfa, which is heated during processing, and low-moisture silage, which undergoes more heating and less proteolysis in the silo than higher-moisture silage (Muck, 1991), supplied more RUP and more intestinally absorbable amino acids.

Steam-heating alfalfa hay for 47 min at 100 to 110°C was found to reduce ruminal protein degradability, although energy digestibility also was reduced (Broderick et al., 1993b). Feeding this hay as the only forage decreased milk yield in cows fed diets with 81% alfalfa. However, feeding the steam-heated hay to replace unheated hay or alfalfa silage at approximately 25% of dietary DM increased DMI and yield of milk and milk components. Milk yield of cows fed the heated hay diet, which had 17% CP, was comparable to that of cows fed a solvent SBM diet containing 23% CP. Alfalfa hay seemed to be more sensitive to overheating than soybeans, but controlled heat treatment of hay showed promise as a means of protecting the protein from ruminal breakdown.

Feeding higher DM silage with decreased NPN content to lactating cows alters their responsiveness to RUP supplementation. Broderick et al. (1993a) found that, at 33 kg of milk/d and DMI equivalent to 3.8% of BW, supplementing CP as urea or true protein from solvent SBM and meat and bone meal did not influence milk yield in cows fed half their forage as corn silage and half as alfalfa silage with 59% DM. However, supplementation with the true protein mixture significantly improved yield of milk and milk components in cows fed low (39%) DM alfalfa silage. The low and high DM alfalfa silages contained, respectively, 53 and 41% NPN. Feeding low DM alfalfa silage may have supplied sufficient degradable CP and made added NPN of little value, while feeding high-DM alfalfa silage might require additional RDP. True protein supplements, particularly those resistant to ruminal degradation, will be used to greatest advantage on legume silage diets containing high basal levels of NPN.

If proteolysis and NPN formation in the silo can be reduced, then forage protein utilization in lactating dairy cows will be improved. Charmley and Veira (1990) suppressed proteolysis in ensiled alfalfa by a 2-min steam treatment, followed by inoculation to allow normal fermentation. This treatment did not alter NDF or ADIN content of the silage but significantly reduced NPN and increased NAN and microbial N flows at the small intestine (Figure 3). Although not practical for production-sized silage making, a clear advantage was shown for reducing proteolysis in the silo. Nagel and Broderick (1992) wilted third-cutting alfalfa to 38% DM and ensiled it as untreated control (**C**), or treated with 8.2 L/ton formic acid (**F**) or 6.3 L/ton of a commercial product containing 16% formaldehyde (**G**). Reduced silage NPN indicated that treatment G, and particularly treatment F, decreased degradation of alfalfa protein in the silo (Table 3). Multiparous cows were fed diets containing 98.5% of DM from one of these silages, plus minerals, vitamins, and a ketosis preventative. Compared with C, yield of milk and fat was increased 3.3 and .20 kg/d with F and G; protein secretion increased .11 kg/d on F and .06 kg/d on G (Table 3). This is interesting because European workers have reported that, although useful

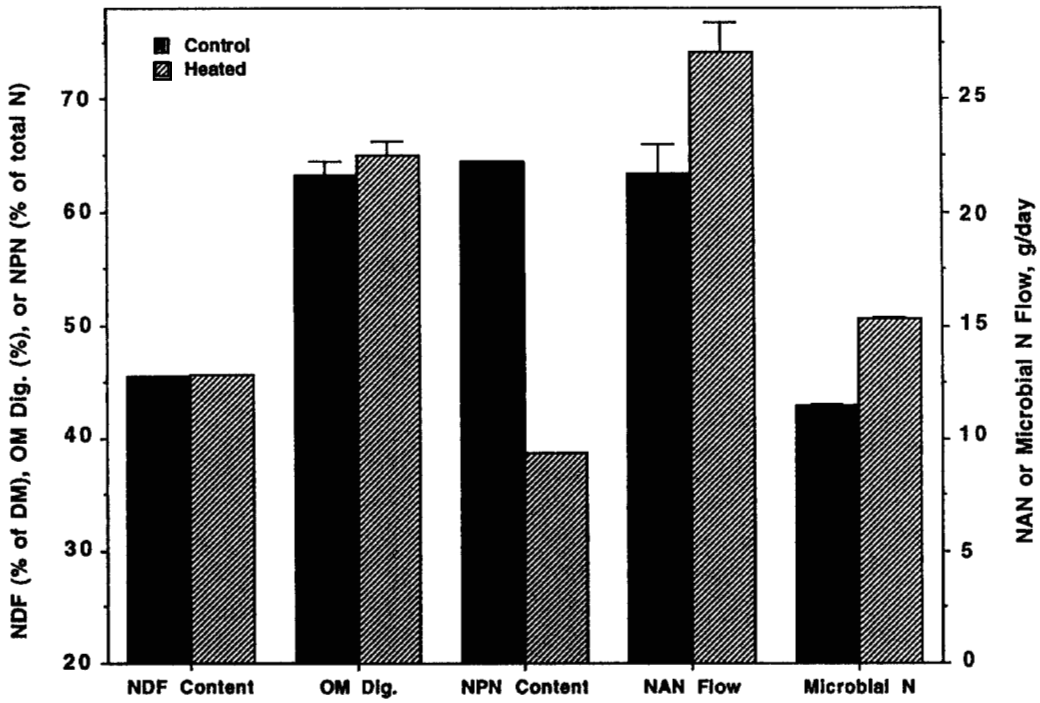

Figure 3. Comparison of NDF and nonprotein N (NPN) content of untreated alfalfa silage (Control) or alfalfa silage ensiled after being heated 2 min at 100°C to suppress proteolysis (Heated), and effect of feeding these silages as the sole diet to sheep on organic matter digestibility (OM Dig.), and nonammonia N (NAN Flow) and microbial N flows at the abomasum (Charmley and Veira, 1990).

for direct-cut silage, formic acid was ineffective when applied to wilted silage (McDonald et al., 1991). Subsequently, replacing 5% of silage DM with FM was found to have no effect on milk yield on any diet or on milk protein content on silage F, but FM increased milk protein by .1 percentage unit on silages C and G.

The performance of lactating cows fed all their forage as either alfalfa silage (**AS**) or alfalfa hay (**AH**) was compared in two trials (Broderick, 1995). Protein adequacy of these diets was assessed from responsiveness of cows to the high-RUP protein source FM: greater milk and protein yields with FM were taken to mean that the diet without FM supplement provided less AP. Alfalfa was harvested from alternate windrows as either 40% DM silage or hay in small rectangular bales and fed to 20 (four with ruminal cannulas) multiparous cows. Diets contained an average (DM basis) 67% alfalfa, 30% high-moisture corn, 1.3% mineral and vitamin supplements, and approximately 1.6 Mcal of NE_l/kg of DM. In Trial 1, alfalfa was from the second cutting and contained 35% NDF; AS had 21.2% CP and AH 19.7% CP. In Trial 2, alfalfa was from the first cutting: AS and AH contained, respectively, 19.9 and 16.5% CP and 40 and 41% NDF. Lower CP in AH than in AS reflected the typically greater leaf loss during hay than during silage harvest (Nelson and Satter, 1992a). Mean NPN content was 52% (AS) and 8% (AH) of total N

Table 3. Effect of silage preservation method on production of cows fed diets containing 98.5% of DM from alfalfa silage (Nagel and Broderick, 1992)

Item	Control	Formic acid	Formaldehyde	SEM
Forage CP, % of DM	21.4	20.8	21.1	.4
Silage NPN, % of total N	43.1	29.1	35.5	1.4
DMI, kg/d	18.3	18.2	19.7	1.1
Milk, kg/d	29.2[b]	32.6[a]	32.5[a]	.9
Milk fat, kg/d	1.10[b]	1.30[a]	1.30[a]	.05
Milk protein, kg/d	.81[b]	.92[a]	.87[ab]	.03
Milk N/N intake	.206	.243	.208	—

[a,b]Means in rows with different superscripts differ ($P < .05$).

in both trials. Two of the diets in each trial had no supplemental protein, whereas the other two diets were supplemented with 3.0% FM (added to replace DM from high-moisture corn). Mean performance data from the two trials are given in Table 4. Cows had lower DMI and lost weight on AS without FM. Fat yield was lower on the AS and AH diets than on AS plus FM; AH plus FM was intermediate. Generally, yields of milk, protein, and solids-not-fat were lower on AS without FM than on the other three diets; FM increased mean protein yield 100 g/d on AS and 30 g/d on AH. Greater energy digestibility in AS (Nelson and Satter, 1992b) may account for the increased milk yield, after correction of protein inadequacy by adding FM, because apparent digestibility of DM, NDF, and ADF were greater on AS (data not shown).

Efficiency of utilization of dietary N was greatest on AH; supplementation with FM actually reduced N efficiency (Table 4). Concentrations of ruminal ammonia and total amino acids were greater at all times after feeding AS; ammonia averaged 15.4 and 8.1 m*M* on AS and AH diets, respectively. Satter and Slyter (1974) reported that ammonia levels in excess of about 4 m*M* were of no value in stimulating net microbial protein production in vitro. In vitro results (V. D. Peltekova, 1994, unpublished data) indicated that the AS and AH fed in this trial had similar contents of RUP but that microbial protein synthesis was 29% greater ($P < .01$) on AH than on AS. Lower concentrations of total amino acids and ammonia on AH suggested that their release during in vivo degradation of AH protein may have been more synchronous with ruminal energy fermentation, thus supporting greater microbial capture of RDP on AH diets than on AS diets. Higher ruminal concentrations of total amino acids and ammonia on AS diets also reflected the greater content of NPN in AS and the 1.6 percentage unit greater CP in those diets.

New developments in mechanization may improve forage harvesting as hay. Koegel et al. (1988) developed an alternative hay-making process involving maceration (extensive shredding) of herbage prior to forming it into thin forage mats that were field-dried. Drying rates of the shredded alfalfa mats were three times that for conventionally harvested alfalfa hay (Koegel et al., 1988). Ruminants derived approximately 15% more energy from shredded than from conventional alfalfa forage (Hong et al., 1988). Shredded hay also had a somewhat greater RUP value as estimated from in vitro incubations (Yang et al., 1993). This may have resulted when soluble sugars, released with herbage cell rupture, were spread over the surface of the plant material, thus enhancing the Maillard reaction between protein and sugars during field drying (R. G. Koegel, personal communication).

Providing sufficient fermentable energy for stimulating microbial capture of RDP seems to be critical on forage silage diets. Dhiman and Satter (1994) randomly assigned 45 multiparous and 29 primiparous cows to one of three diets at calving in a complete lactation study. Cows were fed 50% forage and 50% concentrate (DM basis). Forage was: 1) all from alfalfa silage (AS), 2) 2/3 from AS and 1/3 from corn silage (**CS**), or 3) 1/3 from AS and 2/3 from CS. Dietary NE_l was kept equal by greater fat supplementation of AS diets, but ruminal fermentable energy, presumably, was greater with higher levels of corn silage. Dietary CP content was in proportion to AS content: 18.6, 17.5, and 16.6% in diets 1, 2, and 3, respectively. Milk yield was numerically greatest for both multiparous and primiparous cows on diet 2 (2/3 AS and 1/3 CS); yield on diet 2 for multiparous cows was 577 and 146 kg of milk/lactation greater than on diets 1 and 3, respectively. Ruminal ammonia was lower on CS-containing diets. These results indicated that replacing AS with CS such that dietary CP was 1.1 or 2.0 percentage units lower did not reduce milk yield.

In vitro incubations (C. Agca, unpublished data) indicated that grinding of high-moisture corn (**HMC**) significantly improved ammonia uptake and presumably protein synthesis by mixed ruminal microbes.

Table 4. Effect of supplementing alfalfa silage or hay diets with fish meal on intake, body weight gain, and yield of milk and milk components (Broderick, 1995)[a]

Item	AS	AH	AS+FM	AH+FM	Prob.[b]
Dietary CP, %	17.1	15.4	18.6	17.0	—
DMI, kg/d	22.3[d]	24.0[c]	23.3[cd]	24.2[c]	.005
BW change, kg/d	−.39[d]	.45[c]	.08[cd]	.49[c]	<.001
Milk, kg/d	35.3[d]	36.1[cd]	37.4[c]	36.9[c]	.016
Fat, %	3.44	3.28	3.42	3.31	.405
Fat, kg/d	1.20[d]	1.18[d]	1.28[c]	1.22[cd]	.018
Protein, %	2.96[d]	3.05[c]	3.06[c]	3.07[c]	.013
Protein, kg/d	1.04[e]	1.10[d]	1.14[c]	1.13[cd]	<.001
SNF, %	8.54	8.57	8.56	8.62	.385
SNF, kg/d	3.01[e]	3.09[d]	3.20[c]	3.18[c]	.013
Milk protein N/N Intake	.268	.289	.257	.269	—

[a]AS = alfalfa silage; AH = alfalfa hay; FM = fish meal; DMI = DM intake; BW = body weight; SNF = solids-not-fat.
[b]Probability of a significant treatment effect.
[c,d,e]Means in rows with different superscripts differ ($P < .05$).

Effectiveness of cracked corn (**CC**), ground HMC, and citrus pulp (**CiP**) as carbohydrate sources for stimulating utilization of the NPN in alfalfa silage was assessed in lactating dairy cows fed diets containing (DM basis) 50% alfalfa silage, 10% grass silage, and 28% NDF (Mertens et al., 1994). Six different concentrate mixes were fed for 12 wk each to eight multiparous cows: 1) 39% CC; 2) 39% HMC; 3) 19% CiP plus 19% HMC; diets 4, 5, and 6 were the same as diets 1, 2, and 3, respectively, except 12% expeller SBM replaced equivalent amounts of carbohydrate DM to assess protein yield response to RUP. Supplementation with expeller SBM increased mean protein yield by 109, 67, and 130 g/d, respectively, for CC, HMC, and CiP plus HMC. Greater protein yield responses to RUP on CC and CiP plus HMC indicated that these carbohydrate sources were not as effective as ground HMC for stimulating microbial utilization of alfalfa silage NPN. Similar experimental techniques could be used to identify carbohydrate sources and grain treatments that would give rise to improved N efficiency on forage-based diets. Note that when high-concentrate diets are fed to lactating dairy cows, dietary fiber should be maintained at approximately 28% NDF to prevent low pH and ruminal dysfunction.

Most of the research summarized above was from trials conducted with alfalfa silage and hay. Although we had found that in vitro ruminal degradability of protein in alfalfa silage and hay were similar (V. D. Peltekova, unpublished data), other in vitro studies indicated that the protein in standing alfalfa forage was more degradable than that in alfalfa hay (Broderick et al., 1992). This suggests that the advantages of reducing ruminal protein degradation in grazed forages might be even greater than in harvested forages. Rogers et al. (1980) reported that supplementing lactating cows fed fresh cut ryegrass-white clover herbage with 1.0 kg/d of formaldehyde-treated casein increased milk, protein, and fat yields by 2.0, .07, and .05 kg/d, respectively. This response was similar to those obtained with FM supplementation in the trials discussed above in cows fed alfalfa silage.

Improving the Nutritional Value of Legume Forage Proteins

The foregoing discussion leads to consideration of ways that legume forages might be altered to improve the nutritional value of their proteins for productive ruminants: 1) reduction in both the rate and extent of ruminal protein degradability; 2) reduction or proteolysis and NPN formation in the silo; and 3) increased microbial protein formation in the rumen. Clearly, the three are interrelated. For example, reducing formation of NPN in the silo would reduce excessive ammonia formation in the rumen. Also, the rate of degradation of silage proteins not converted to NPN in the silo would be more likely to be in synchrony with microbial protein synthesis and, thus, improve microbial capture of RDP.

The distribution of intact proteins in forage leaves is summarized in Table 5 (Mangan, 1982). Nearly two-thirds of total leaf protein is found in ribisco, the CO_2 fixing enzyme ribulose-1,6-bisphosphate carboxylase, plus fraction 2 proteins, which are a complex mixture of enzymatic proteins. Another 30% of intact plant proteins are found in the membranes of the plant; proteins associated with the plant's nucleic acids and elastin represent a small fraction of intact leaf proteins. It is not likely that any of these proteins may be tampered with without altering the metabolic or genetic machinery of the plant. A more promising tack might be to add an exogenous compound to reduce autolytic or microbial proteolysis rather than trying to change these functional proteins themselves.

McDonald et al. (1991) have summarized the steps of autolytic proteolysis that occur during wilting and ensiling. Briefly, as soon as the plant is cut, rupture of plant membranes begins releasing the proteases from the vacuoles and the enzymatic and membrane (substrate) proteins from the plant organelles. Activation of these proteases accelerates the process of autolysis. The pH and temperature optima for forage plant proteases are approximately 5.5 and 45 to 55°C, respectively, although these proteases represent a complex mixture of enzymes (McKersie, 1981). During wilting, forage protein autolysis proceeds at rates largely controlled by access of substrate protein to protease; as long as the forage material can respire in the presence of O_2, forage plants organelle membranes remain largely intact and protein breakdown will be restricted. However, the O_2 in the silage mass will be quickly depleted after ensiling and the forage plant organelles degrade, exposing their proteins to increased autolytic attack. The elevated temperature and moderate pH decline will enhance the rate of proteolysis; proteolysis slows markedly at pH 3.8 to 4.0, but does not cease completely. The extent of protein breakdown to NPN in the silo is primarily controlled by availability of substrate (McKersie and Buchanan-Smith, 1982). Some advantage can be gained by more rapid pH drop, such as occurs with

Table 5. Distribution of intact proteins in forage leaves (Mangan, 1982)

Protein fraction	Proportion[a]
Ribisco (fraction 1 protein)	32–40%
Fraction 2 protein	~25%
Thylakoid (membrane) proteins	~25%
Mitochondrial proteins	<5%
Nucleoproteins	1–2%
Extensin	"low"

[a]Proportions of intact proteins. Free amino acids and other small molecular weight compounds represent 10 to 20% of total N.

inoculation with lactic acid bacteria or added fermentable sugars, but these effects are small in terms of preserved forage protein (Muck, 1991).

Carpintero et al. (1979) reported on the magnitude of changes in NPN during wilting of ryegrass-clover forage. As forage DM increased from 17 to 46%, after 48 h of "good" field drying conditions, NPN increased from 8 to 18% of total N. Formation of NPN was greater under poor drying conditions. Under experimentally imposed "moist" drying conditions, NPN content of forage was 25 and 31% of total N at 20 and 38% DM. Proteolysis will proceed in the swath as long as DM content of the forage remains less than approximately 70% (Muck, 1991). These data suggest that approximately one-fourth to one-third of the NPN found in silage is already present before ensiling.

The major forage proteins do not survive autolysis during wilting and ensiling. Fairbairn et al. (1988) reported on the changes in ribisco and NPN content of direct-cut alfalfa silage. The pattern of release of ammonia, total amino acids, and total NPN was typical for well-preserved alfalfa silage; NPN represented from 35 to 42% of total N from 14 to 90 d after ensiling (Figure 4). However, disappearance of ribisco was very rapid, and it was completely degraded in 2 d (inset, Figure 4). Rate and extent of NPN formation in this trial were lower than usual; thus, one may expect that ribisco, and probably other soluble proteins in forages, stand little chance of surviving ensiling under normal conditions.

A trial was conducted to determine the extent to which typical acid treatments reduce proteolysis simply due to pH decrease or whether protease activity also is reduced by acid treatment (Vagnoni et al., 1992). Alfalfa was cut and wilted to 40% DM and ensiled, after inoculation, in small experimental silos as either untreated control or with pH adjusted to 4.0 with formic acid, sulfuric acid, or trichloroacetic acid (Figure 5). Forage contained about 15% NPN before ensiling; 4 d after ensiling, the silages had reached maximal NPN contents; all of the acid treatments decreased NPN production. Protein breakdown was approximately equal in formic and sulfuric acid-treated silages. However, trichloroacetic acid, which inactivates most enzymes, almost completely suppressed NPN formation (Figure 5). These results clearly indicated that proteolysis continues in silage well after pH has decreased, even to 4.0, regardless of whether the pH drop was due to normal fermentation or acid treatment.

In a novel study, Makoni et al. (1994) observed a reduction in proteolysis with ensiling of alfalfa when silos were flushed daily with a modified atmosphere (3% O_2:20% CO_2: 77% N_2). After 28 d, NPN contents of alfalfa silage treated with the modified atmosphere and formic acid (applied at a rate of 6.0 L/ton, wet basis) were, respectively, 36.5 and 37.2% of total N, compared with 51.0% in untreated silage. The pH in silages treated with modified atmosphere and formic acid were 7.0 and 4.6. The modified atmosphere may

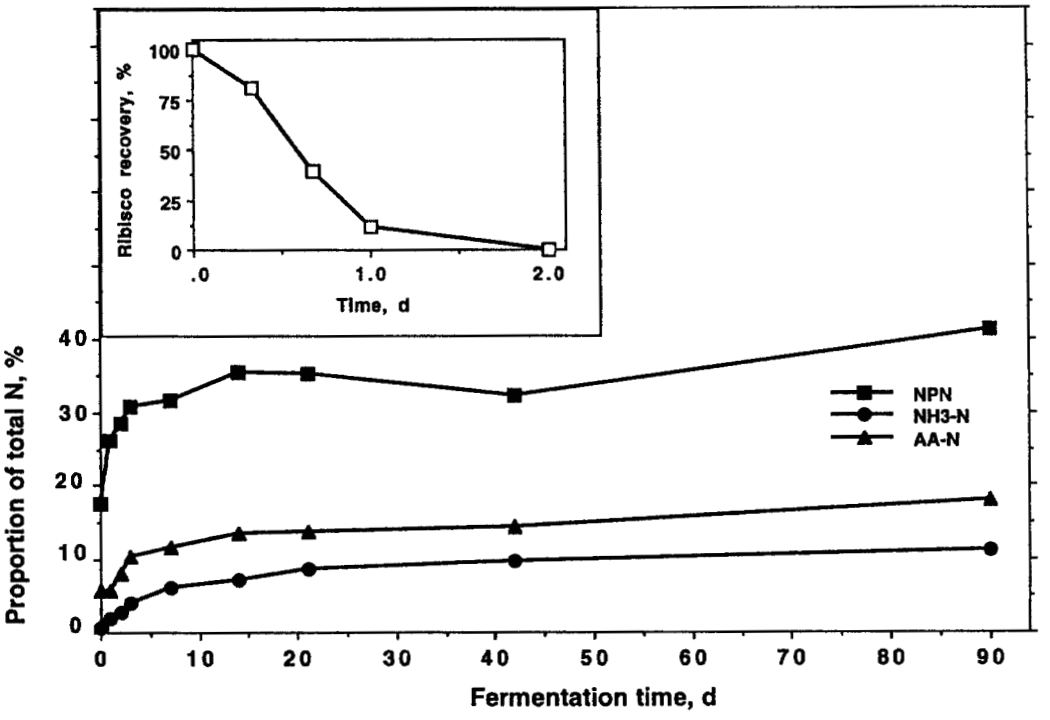

Figure 4. Release of N as ammonia (NH_3 N), total amino acids (AA N) and nonprotein N (NPN), and disappearance of ribisco (inset), with time after ensiling of untreated, direct-cut alfalfa silage (Fairbairn et al., 1988).

have prevented degradation of plant organelles, thus restricting the release and mixing of proteases and substrate proteins.

There are differences among forage species in the rates of proteolysis after ensiling. Red clover, which does not contain tannins, produces silage with typically much less NPN than alfalfa (Papadopoulos and McKersie, 1983). Results of Jones et al. (1995) are typical. From 0 to 7 d after ensiling, the NPN content of red clover forage increased from 14 to 48% of total N, whereas alfalfa forage increased from 16 to 80% of total N over the same time period. Rates of proteolysis of extracts from fresh alfalfa and red clover forage are shown in Figure 6 (Jones et al., 1995). Free amino acid release in alfalfa extract was approximately five times greater than that in red clover extract. Mixing red clover and alfalfa extracts substantially decreased proteolysis, whereas mixing alfalfa extract with boiled red clover extract yielded free amino acid release that was slightly more rapid than alfalfa alone (Figure 6). Jones et al. (1995) explained these results based on the presence in red clover of a polyphenol oxidase system that produces phenolic compounds that inhibit proteolysis during ensiling.

Albrecht and Muck (1991) ensiled direct-cut forage material from a number of legume species over 2 yr in small experimental silos; NPN proportions in silages from 1 yr are plotted in Figure 7. They found that silage made with legumes containing condensed tannins (e.g., sainfoin, sericea lespedeza, and *Lotus pedunculatus*) and one non-tannin legume, red clover, all had lower NPN proportions than alfalfa silage (Figure 7). Only limited success at reducing NPN was obtained by ensiling a blend of sainfoin and alfalfa (R. E. Muck, unpublished data). Clearly, further research is warranted on the use of alternative legumes for ensiling that could reduce NPN formation in the silo.

Variation in ruminal protein degradation rate among several forage legumes was used to determine the degree to which degradability was reduced by tannins and other factors (Broderick and Albrecht, 1994). A range of legume species adapted to temperate regions were grown and harvested during 2 yr and analyzed for condensed tannins and for rates of in vitro ruminal protein degradation, estimated using a Michaelis-Menten approach, and for proportions of ruminal protein escape (RUP values). Protein degradation rates and ruminal escapes in forages harvested the 1st yr ranged from .36/h and 13% for crown vetch to .047/h and 54% for a cultivar of *Sericea lespedeza*, and, in the 2nd yr, from .26/h and 18% for alfalfa and a low-tannin accession of birdsfoot trefoil to .038 to .040/h and 58 to 59% for two sainfoin accessions and three *Sericea lespedeza* accessions. Generally, reduced degradation rate and increased ruminal escape were proportional to tannin concentration in forage produced over the 2 yr (Figure 8). A quadratic regression of estimated Michaelis-Menten rates on tannin concentration was fit for all accessions with detectable levels of condensed tannin (r^2 = .924;

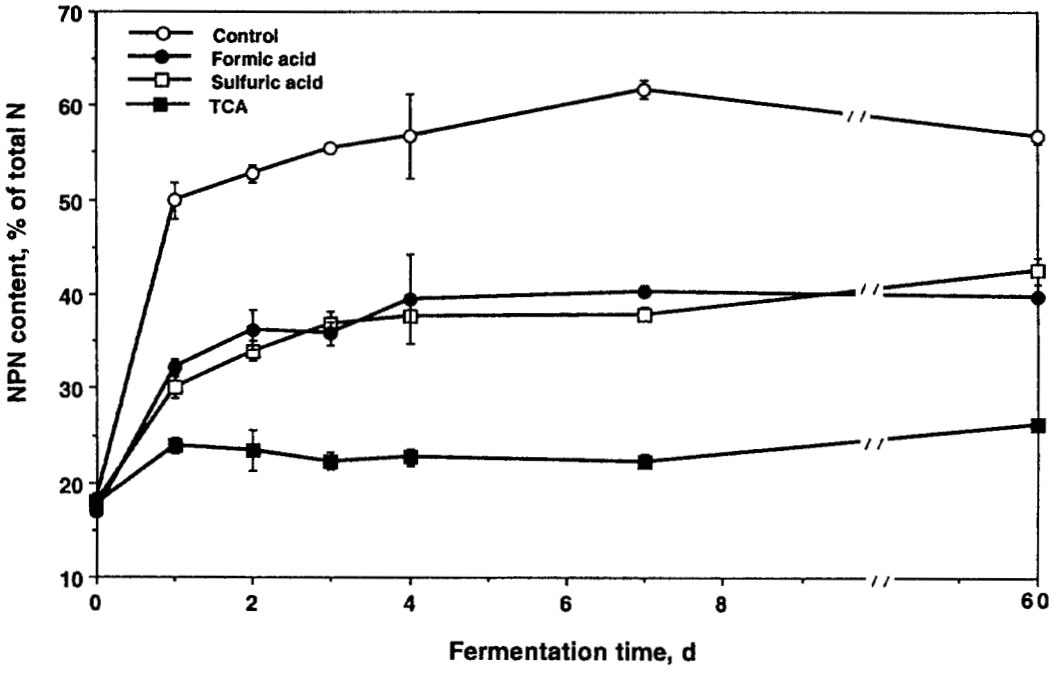

Figure 5. Formation of nonprotein N (NPN) with time after ensiling of untreated alfalfa silage (Control) and alfalfa forage adjusted to pH 4.0 at ensiling using formic acid, sulfuric acid, or trichloroacetic acid (TCA) (Vagnoni et al., 1992).

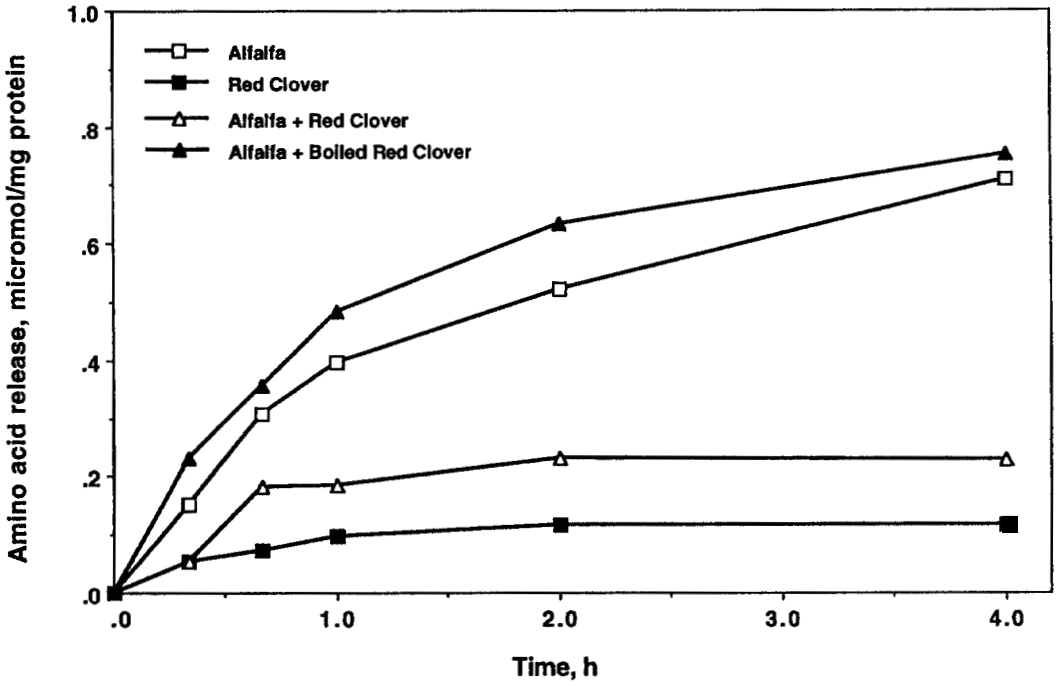

Figure 6. Rates of proteolysis, expressed as release of free amino acids, in buffer extracts from fresh alfalfa (Alfalfa), fresh red clover (Red Clover), a mixture of extracts from fresh alfalfa plus red clover (Alfalfa + Red Clover), and a mixture of fresh alfalfa extract plus boiled red clover extract (Alfalfa + Boiled Red Clover) (Jones et al., 1995).

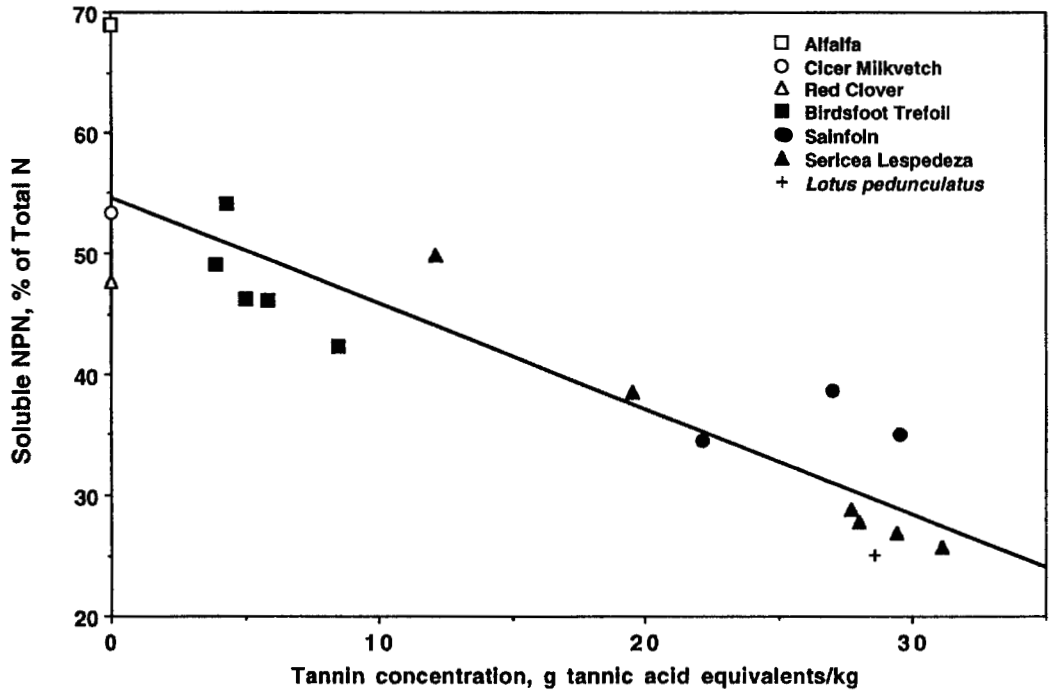

Figure 7. Regression of soluble nonprotein N (NPN), as a proportion of total N (Y), on condensed tannin concentration (X) 45 d after ensiling samples of seven legume forage species. Mean data from 1988 only (Albrecht and Muck, 1991) were graphed. $Y = 54.8 - .875\ X$; $r^2 = .799$, $P < .01$.

Figure 8). Solving this equation for the tannin concentration for which the estimated degradation rate was minimal yielded an estimate of 27 g of tannic acid equivalents/kg of DM, corresponding to a degradation rate of .048/h and a ruminal protein escape of 56%. However, several of the forages not containing condensed tannins, notably red clover and kura clover, had protein degradabilities comparable to those of forages with low levels of tannin. The possible role of the polyphenol oxidase system in red clover already has been discussed. These data suggested that differences in ruminal protein degradation among forage legumes is only partly explained by tannin concentrations. It should be noted that small genetic differences in proteolysis in silage (Bowley and McKersie, 1987) and in ruminal protein degradability were found in alfalfa germplasm (Broderick and Buxton, 1991).

In the future, genetic selection or genetic engineering may be used to produce forages with improved protein efficiency. Use of biotechnology to add tannins to non-tannin forages may have potential for reducing protein breakdown both in the silo and in the rumen. However, use of genetic engineering to inject genes for resistant proteins into forages may have less application (Tabe et al., 1995). Low levels of expression of genes coding for certain high-sulfur proteins have been achieved in alfalfa (e.g., Spencer et al., 1988; Schroeder et al., 1991). These proteins are soluble but resistant to ruminal degradation, partly because of the extensive disulfide cross-linking (Wallace, 1983). Being soluble, these proteins will efflux from the rumen with the liquid phase more rapidly than will insoluble proteins, which travel with the solid phase (Broderick et al., 1991). If sufficient levels of expression were obtained in a forage, then their high cystine contents would make these proteins potentially valuable RUP sources for the wool-producing sheep grazing the altered forage. However, this approach probably will not prove successful for use in dairy cattle because expression of the foreign genes may be too low. To add 1% of a resistant protein to a dairy cow diet containing 50% alfalfa forage DM would require expression of the gene coding for that protein at 2% of the DM or 10% of the protein in alfalfa. This level of expression seems unlikely (Tabe et al., 1995). Furthermore, introducing resistant proteins into forages does not change the degradable nature of the proteins already present in forages. Moreover, supplementing resistant proteins in non-grazing ruminants can be done much more inexpensively by feeding heat-treated proteins such as roasted soybeans or naturally resistant proteins such as FM or blood meal.

A number of potential solutions are available to improve efficiency of utilization of forage proteins in dairy cows and other ruminants. Currently, ration formulation approaches may be used to feed more RUP or to correct for excessive degradation of forage proteins. If cows are fed a minimal amount of effective

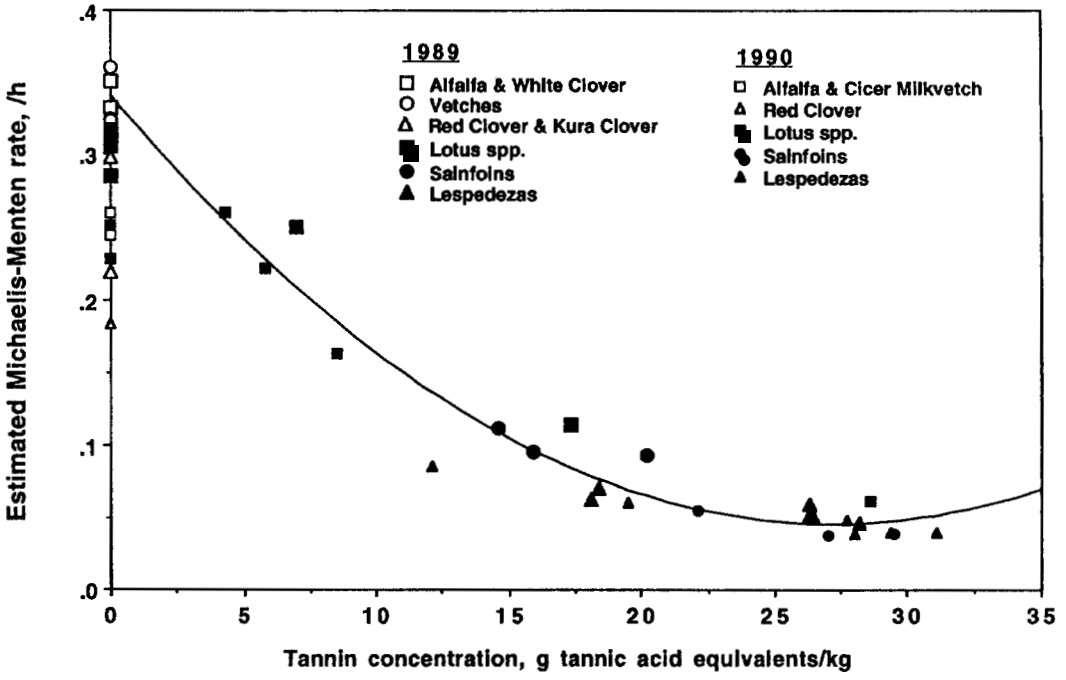

Figure 8. Quadratic regression of estimated Michaelis-Menten degradation rates (Y) on condensed tannin concentrations (X). Michaelis-Menten rates estimated for accessions from 11 species of legumes from 2 yr (1989 and 1990). $Y = .340 - .0216\ X + .0004\ X^2$; $r^2 = .924$; $P < .001$. Estimated Michaelis-Menten rate was minimal (.048/h) at 27 g of tannic acid equivalents/kg of DM (Broderick and Albrecht, 1994).

fiber (approximately 28% NDF in the DM), then maximizing fermentable energy in the ration will maximize microbial capture of degraded forage protein for protein synthesis in the rumen. This may be accomplished by feeding greater amounts of concentrates, more finely grinding the concentrate portion of the ration, and replacing part of the forage, such as alfalfa silage, with higher-energy forage, such as corn silage. Red clover silage, which has lower NPN content, may be used to partially replace high-NPN silages made from alfalfa or other legumes. Although not adapted to being widely cultured in North America, certain tannin-containing legumes have potential for producing forage with lower protein degradation in the silo and in the rumen. Slower ruminal degradation of protein in alfalfa hay, and possibly hay protein generally, may result in more efficient utilization of its RDP for microbial protein formation. Hay harvesting techniques that increase drying rate, reduce leaf loss, and improve mechanization are under development; these may allow capitalizing on the greater efficiency of protein utilization obtained with feeding forage as hay than silage.

Future research should focus on methods for reducing NPN formation in legume silages. Increased rate and extent of pH drop in the silo will decrease proteolysis. This could be accomplished by increasing fermentable carbohydrates in legume forages. Reduced proteolysis in the silo would attend the insertion of condensed tannins into alfalfa or other forages through genetic engineering. These forages would have protein that was more efficiently utilized when either grazed or harvested. Biotechnological techniques might be used to obtain expression of genes coding for protease inhibitors, perhaps targeted for secretion into the vacuole or other plant organelle where their release, after membrane disruption during wilting, ensiling, or with chewing, might inhibit proteolysis in the silo or in the rumen. Inserting genes coding for resistant proteins into forages may have limited applicability. Better understanding of the processes by which plant proteins and proteases are released from forage material as it undergoes ensiling may lead to methodologies for stabilizing plant organelles or otherwise suppressing the autolysis process.

Implications

Inefficient utilization of legume forage protein, and the overfeeding of protein supplements that it necessitates, lead to excessive N losses to the environment from dairy enterprises. Feeding more concentrate, or more finely ground concentrate, while maintaining minimum effective fiber, is required to maximize capture of the degraded forage protein for ruminal protein synthesis. Because of lower proportions of nonprotein N, the N in red clover silage is used more efficiently than that in alfalfa silage. Lower degradation in the silo and the rumen makes the protein in tannin-containing legumes more effectively utilized in ruminants; however, most tannin-containing legumes are not widely adapted to culture in North America. Slower ruminal degradation of hay protein increases its efficiency of N incorporation into microbial protein.

Literature Cited

Albrecht, K. A., and R. E. Muck. 1991. Proteolysis in ensiled forage legumes that vary in tannin concentration. Crop Sci. 31:464.

Argyle, J. L., and R. L. Baldwin. 1989. Effects of amino acids and peptides on rumen microbial growth yields. J. Dairy Sci. 72: 2017.

Beever, D. E. 1982. Protein utilization from pasture. In: T. W. Griffiths and M. F. Maguire (Ed.) Forage Protein Conservation and Utilisation. p 99. Commission of the European Communities, Dublin, Ireland.

Bergen, W. G. 1979. Free amino acids in blood of ruminants–physiological and nutritional regulation. J. Anim. Sci. 49:1577.

Bowley, S. R., and B. E. McKersie. 1987. Genetic variance of proteolytic activity in alfalfa herbage. Can. J. Plant Sci. 67:159.

Broderick, G. A. 1985. Alfalfa silage or hay versus corn silage as the sole forage for lactating dairy cows. J. Dairy Sci. 68:3262.

Broderick, G. A. 1992. Relative value of fish meal versus solvent soybean meal for lactating dairy cows fed alfalfa silage as sole forage. J. Dairy Sci. 75:174.

Broderick, G. A. 1995. Performance of lactating dairy cows fed either alfalfa silage or alfalfa hay as the sole forage. J. Dairy Sci. 78: 320.

Broderick, G. A., S. M. Abrams, and C. A. Rotz. 1992. Ruminal in vitro degradability of protein in alfalfa harvested as standing forage or baled hay. J. Dairy Sci. 75:2440.

Broderick, G. A., and K. A. Albrecht. 1994. Ruminal in vitro degradation of protein in tannin free and tannin containing forage legume species. J. Dairy Sci. 77(Suppl. 1):385 (Abstr.).

Broderick, G. A., and D. R. Buxton. 1991. Genetic variation in alfalfa for ruminal protein degradability. Can. J. Plant Sci. 71:755.

Broderick, G. A., W. M. Craig, and D. B. Ricker. 1993a. Urea versus true protein as supplement for lactating dairy cows fed grain plus mixtures of alfalfa and corn silages. J. Dairy Sci. 76:2266.

Broderick, G. A., and N. R. Merchen. 1992. Markers for quantifying microbial protein synthesis in the rumen. J. Dairy Sci. 75:2618.

Broderick, G. A., D. B. Ricker, and L. S. Driver. 1990. Expeller soybean meal and corn by-products versus solvent soybean meal for lactating dairy cows fed alfalfa silage as sole forage. J. Dairy Sci. 73:453.

Broderick, G. A., R. J. Wallace, and E. R. Ørskov. 1991. Control of rate and extent of protein degradation. In: T. Tsuda, Y. Sasaki, and R. Kawashima (Ed.) Physiological Aspects of Digestion and Metabolism in Ruminants. p 541. Academic Press, Orlando, FL.

Broderick, G. A., J. H. Yang, and R. G. Koegel. 1993b. Effect of steam heating alfalfa hay on utilization by lactating dairy cows. J. Dairy Sci. 76:165.

Cardorniga, C., and L. D. Satter. 1993. Protein versus energy supplementation of high alfalfa silage diets for early lactation cows. J. Dairy Sci. 76:1972.

Carpintero, C. M., A. R. Henderson, and P. McDonald. 1979. The effect of some pretreatments on proteolysis during the ensiling of herbage. Grass Forage Sci. 34:311.

Cecava, M. J., N. R. Merchen, L. C. Gay, and L. L. Berger. 1990. Composition of ruminal bacteria harvested from steers as influenced by dietary energy level, feeding frequency, and isolation techniques. J. Dairy Sci. 73:2480.

Charmley, E., and D. M. Veira. 1990. Inhibition of proteolysis in alfalfa silages using heat at harvest: Effects on digestion in the rumen, voluntary intake and animal performance. J. Anim. Sci. 68:2042.

Dhiman, T. R., C. Cadorniga, and L. D. Satter. 1993. Protein and energy supplementation of high alfalfa silage diets during early lactation. J. Dairy Sci. 76:1945.

Dhiman, T. R., and L. D. Satter. 1994. Milk yield and rumen fermentation measurements in cows fed diets containing different proportions of alfalfa and corn silage J. Dairy Sci. 77(Suppl. 1):222 (Abstr.).

Fairbairn, R., I. Alli, and B. E. Baker. 1988. Proteolysis associated with the ensiling of chopped alfalfa. J. Dairy Sci. 71:152.

Faldet, M. A., and L. D. Satter. 1991. Feeding heat-treated full fat soybeans to cows in early lactation. J. Dairy Sci. 74:3047.

Hong, B. J., G. A. Broderick, R. G. Koegel, K. J. Shinners, and R. J. Straub. 1988. Effect of shredding alfalfa on cellulolytic activity, digestibility, rate of passage, and milk production. J. Dairy Sci. 71:1546.

Hristov, A., and G. A. Broderick. 1994. In vitro determination of ruminal protein degradability using [^{15}N]-ammonia to correct for microbial nitrogen uptake. J. Anim. Sci. 72:1344.

Jones, B. A., R. E. Muck, and R. D. Hatfield. 1995. Red clover extracts inhibit legume proteolysis. J. Sci. Food Agric. 67 (In press).

Koegel, R. G., K. J. Shinners, F. J. Fronczak, and R. J. Straub. 1988. Prototype for production of fast-drying forage mats. Appl. Eng. Agric. 4:126.

Makoni, N. F., G. A. Broderick, and R. E. Muck. 1994. Effect of modified atmosphere and formic acid on the extent of proteolysis in ensiled alfalfa. J. Dairy Sci. 77(Suppl. 1):275 (Abstr.).

Mangan, J. L. 1982. The characterization of forage protein. In: T. W. Griffiths and M. F. Maguire (Ed.) Forage Protein Conservation and Utilisation. p 1. Commission of the European Communities, Dublin, Ireland.

McDonald, P., A. R. Henderson, and S.J.E. Heron. 1991. The Biochemistry of Silage. John Wiley & Sons, New York.

McKersie, B. D. 1981. Proteinases and peptidases in alfalfa herbage. Can. J. Plant Sci. 61:53.

McKersie, B. D., and J. Buchanan-Smith. 1982. Changes in the levels of proteolytic enzymes in ensiled alfalfa forage. Can. J. Plant Sci. 62:111.

Merchen, N. R., and L. D. Satter. 1983. Changes in nitrogenous compounds and sites of digestion of alfalfa harvested at different moisture contents. J. Dairy Sci. 66:789.

Mertens, D. R. 1987. Predicting intake and digestibility using mathematical models of ruminal function. J. Anim. Sci. 64: 1548.

Mertens, D. R., G. A. Broderick, and R. Simons. 1994. Efficacy of carbohydrate sources of improving utilization of N in alfalfa silage. J. Dairy Sci. 77(Suppl. 1):240 (Abstr.).

Muck, R. E. 1987. Dry matter level effects on alfalfa silage quality. 1. Nitrogen transformations. Trans. Am. Soc. Agric. Eng. 30:7.

Muck, R. E. 1991. Silage fermentation. In: G. Zeikus and E. A. Johnson (Ed.) Mixed Cultures in Biotechnology. p 171. McGraw-Hill, New York.

NRC. 1985. Ruminant Nitrogen Usage. National Academy Press, Washington, DC.

NRC. 1989. Nutrient Requirements of Dairy Cattle (6th Rev. Ed.). National Academy Press, Washington, DC.

Nagel, S. A., and G. A. Broderick. 1992. Effect of formic acid or formaldehyde treatment of alfalfa silage on nutrient utilization by dairy cows. J. Dairy Sci. 75:140.

Nelson, W. F., and L. D. Satter. 1992a. Impact of alfalfa maturity and preservation method on milk production by cows in early lactation. J. Dairy Sci. 75:1562.

Nelson, W. F., and L. D. Satter. 1992b. Impact of stage of maturity and method of preservation of alfalfa on digestion in lactating dairy cows. J. Dairy Sci. 75:1571.

Owens, F. N., and A. L. Goetsch. 1986. Digesta passage and microbial protein synthesis. In: L. P. Milligan, W. L. Grovum, and A. Dobson (Ed.) Control of Digestion and Metabolism in Ruminants. p 196. Prentice-Hall, Englewood Cliffs, NJ.

Papadopoulos, Y. A., and B. D. McKersie. 1983. A comparison of protein degradation during wilting and ensiling of six forage species. Can. J. Plant Sci. 63:903.

Roffler, R. E., and L. D. Satter. 1985. Evaluation of alfalfa preserved as high-moisture silage, low-moisture silage or dehydrated pellets. Research Summaries of the U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI. p 7.

Rogers, G. L., R.H.D. Porter, T. Clarke, and J. A. Stewart. 1980. Effect of protected casein supplements on pasture intake, milk yield and composition of cows in early lactation. Aust. J. Agric. Res. 31:1147.

Satter, L. D., and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. Br. J. Nutr. 32:199.

Schroeder, H. E., M.R.I. Khan, W. R. Knibb, D. Spencer, and T.J.V. Higgins. 1991. Expression of a chicken ovalbumin gene in three lucerne cultivars. Aust. J. Plant Physiol. 18:495.

Spencer, D., T.J.V. Higgins, M. Freer, H. Dove, and J. B. Coombe. 1988. Monitoring the fate of dietary proteins in rumen fluid using gel electrophoresis. Br. J. Nutr. 60:241.

Stokes, S. R., W. H. Hoover, T. K. Miller, and R. P. Manski. 1991. Impact of carbohydrates and protein levels on bacterial metabolism in continuous culture. J. Dairy Sci. 74:860.

Strobel, H. J., and J. B. Russell. 1986. Effect of pH and energy spelling on bacterial protein synthesis by carbohydrate-limited cultures of mixed rumen bacteria. J. Dairy Sci. 69:2941.

Tabe, L., M.R.I. Khan, T. Wardley-Richardson, S. Craig, and T. J. Higgins. 1995. A biotechnological approach to improving the nutritive value of alfalfa. J. Anim. Sci. 73:2752.

Tamminga, S. 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75:345.

Vagnoni, D. B., R. E. Muck, and G. A. Broderick. 1992. Preservation of protein in high moisture alfalfa silage by direct acidification. J. Dairy Sci. 75(Suppl. 1):207 (Abstr.).

Voss, V. L., D. Stehr, L. D. Satter, and G. A. Broderick. 1988. Feeding lactating dairy cows proteins resistant to ruminal degradation. J. Dairy Sci. 71:2428.

Wallace, R. J. 1983. Hydrolysis of ^{14}C-labelled proteins by rumen microorganisms and by proteolytic enzymes prepared from rumen bacteria. Br. J. Nutr. 50:345.

Wallace, R. J. 1994. Amino acid and proteins synthesis, turnover, and breakdown by ruminal microorganisms. In: J. M. Asplund (Ed.) Principles of Protein Nutrition of Ruminants. p 71. CRC Press, Boca Raton, FL.

Yang, J. H., G. A. Broderick, and R. G. Koegel. 1993. Effect of heat treating alfalfa hay on chemical composition and ruminal in vitro protein degradation. J. Dairy Sci. 76:154.